

In re Application of:
Waterman, *et. al.*
Application No.: 10/060,844
Filed: January 29, 2002
Page 4

PATENT
ATTY. DOCKET NO.: UCI1160-1

REMARKS

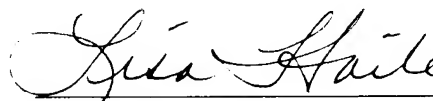
By the present amendment, Applicants respectfully request incorporation of the attached Sequence Listing into the application. The specification has been amended to insert "SEQ ID NO:1" into [0019] on page 6. Accordingly, no new matter has been added by the submission of the present amendments.

CONCLUSION

If the Examiner would like to discuss any of the issues raised in this Amendment or the attached sequence listing, Applicants' representative can be reached at (858) 677-1456.

Respectfully submitted,

Date: June 14, 2002



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EXHIBIT A

MARKED-UP COPY SHOWING AMENDMENTS TO SPECIFICATION AND CLAIM

In the Specification:

Please amend the specification as follows:

[0019] Figure 4 shows LEF-1DN can repress activation of reporter gene expression by β -catenin. The LEF/TCF reporter plasmid TOPtk was co-transfected into Jurkat T lymphocytes with increasing amounts of an expression vector for Δ NLEF (amounts are indicated in micrograms of co-transfected plasmid), a truncated form of LEF1 similar in structure to LEF-1DN (aa67-399). The LEF1 promoter is activated by TCF1- and TCF4- β -catenin complexes in 2017 T lymphocytes. a, A luciferase reporter gene driven by the LEF1 promoter (-672, +305) was co-transfected with expression vectors for full length TCF1E or TCF4E and β -catenin. Activation was calculated using equivalent amounts of empty expression vector. TCF1E activated luciferase gene expression 7.0-fold and TCF4E activated 4.6-fold in this representative experiment. Fold activation by TCF1 over 5 replicate experiments is 8 ± 3.65 (SD), for TCF4, $5.6\text{-fold} \pm 3.7$ (SD). Co-transfection of TCF1E and D19 β -catenin, a mutant that cannot bind to LEF/TCF proteins, did not activate the promoter. b, DNAase I footprint analysis of the LEF1 promoter with recombinant LEF1 protein reveals two binding sites downstream of the start site of transcription. The footprints are centered over two close matches to LEF/TCF consensus binding sites (YCTTTGWW): TCTTTGCTTT (+190) (SEQ ID NO:1) and TCTTTGTTC (+283). A fast migrating portion of intact probe obscures the +190 footprint with LEF1 protein in the second panel. Whole cell extracts from Jurkat T lymphocytes (express TCF4, TCF1 and LEF1) but not HeLa cells (little to no LEF/TCF expression) protect the +283 site but not the +190 site. c, Fragments of the LEF1 promoter were cloned into pGL2-enhancer plasmids and tested for activation by TCF1 and β -catenin. The region responsive to TCF/ β -catenin encompasses the downstream LEF/TCF binding sites. Activation of the largest fragment (-672, +305) was 9.2-fold, whereas activation of fragments that delete the +283 LEF/TCF binding site with (-672, +262) or without (-64, +262) the upstream sequences are activated 4.3- and 3.6-fold respectively. Removal of both the +190 and +283 binding sites (to +78) reduces activation to 1.6-fold. d, Transient overexpression of a GFP/APC fusion protein in SW480 cells reduces

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Page 6

PATENT

ATTY. DOCKET NO.: UCI1160-1

LEF1 promoter reporter gene activity (-672, +305) three-fold. The parent construct which expresses only the GFP portion does not inhibit promoter activity. Whole cell extracts from Colo320 cells overexpressing GFP/APC were analyzed by western analysis with b-catenin monoclonal antisera, and LEF/TCF polyclonal antisera (75,000 cell equivalents; inset). A decrease in b-catenin and LEF1 levels is observed, but not a decrease in TCF4 levels (indicated by filled circle).